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## Agriculture Development Fund

# FINAL REPORT

**USING INTERLEUKIN-6 TO IMPROVE MUCOSAL VACCINES**

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# ADF FINAL PROGRESS REPORT

**#ADF97000314**

**"Using Interleukin-6 to Improve Mucosal Vaccines"**

**by**

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Summary ..... 2
Technical Report ..... 2
Background ..... 2
Specific Aims and Milestones: ..... 3
Modification to Specific Aims: ..... 4
Project Activities ..... 4
AIM 1: Develop antibody and ELISPOT assays to detect mucosal antibody (IgA) ..... 4
AIM 2: Determine the dose of BAV-3 expressing gD (antigen of bovine herpesvirus-1) that will elicit a mucosal immune response ..... 5
AIM 3: Construct an adenovirus vector containing the bovine IL6 ..... 6
AIM 4: Develop a bioassay to confirm the expression of IL6 ..... 8
AIM 5 Confirm the expression of bioactive IL6 from the IL6-adenovirus ..... 9
AIM 6 Determine the dose of IL6-adenovirus which will elicit a biologically detectable response without adverse effects ..... 10
AIM 7 Assess the effect of IL6 on the antibody response to a vaccine ..... 12
Conclusions ..... 15
Information of benefit to producers ..... 15
Achievements, Papers, and Presentations ..... 15

Acknowledgement ..... 16

*Figure 1. Detection of Sheep IgA* ..... 4
*Figure 2 Antibody responses to bovine adenovirus expressing gD* ..... 5
*Figure 3. Diagram of construction of bovine adenovirus containing the bovine IL6 gene.* ..... 7
*Figure 4 Ovine Retropharyngeal Lymph Node Cell Response to IL6* ..... 9
*Figure 5 Anti-IL6 Blocks IgA Inducing Effect of Bovine Fibroblast Culture Supernatants* ..... 9
*Figure 6 Production of Bioactive IL6 by BAV3 Vectors* ..... 10
*Figure 7 Induction of the acute phase protein haptoglobin* ..... 11
*Figure 8 IgA Levels In Tracheal Mucous* ..... 12
*Figure 9 Acute phase responses following immunization* ..... 13
*Figure 10 Antibody responses to gD* ..... 14

## SUMMARY

Vaccines that are delivered to the mucosal surfaces, such as the respiratory or gastrointestinal tracts, may be more effective in preventing disease since they stimulate immunity where many viruses and bacteria start their infection. As well, intranasal or oral vaccines have no injection site lesions, improving carcass quality. To improve the immune response to mucosal vaccines, we proposed to administer a cytokine, interleukin 6 (IL6), with the vaccine. IL6 is a natural regulator of the immune system, where it enhances the production of antibodies. **Thus, the objective of this proposal was to prove that delivery of IL6 in conjunction with a vaccine would improve the immune response at mucosal surfaces, such as the respiratory tract.**

A challenge for mucosal vaccines is finding a way to deliver the vaccine to the mucosal site. One option is to use an innocuous bacteria or virus as a vector to carry the gene for the vaccine antigen. The live vector infects the mucosa, producing the antigen at that site. By inserting the cytokine gene into the vector, the cytokine is expressed at the same time and place as the antigen. The bovine adenovirus-3 (BAV3) was used to deliver the vaccine antigen and IL6 in this project. To achieve our objective, we:

- constructed an adenovirus vector containing the bovine IL6 gene
- developed a bioassay to confirm the expression of IL6
- determined the dose of IL6-adenovirus which elicited a biologically detectable response, without adverse effects, in young lambs
- assessed the effect of IL6 on the antibody response to a vaccine antigen.

Although we were successful in constructing the vector, and eliciting some IL-6 dependent responses by administering the BAV3 with the IL6 gene, we did not confirm the hypothesis that coadministration of IL6 with a vaccine would augment the immune response to that vaccine.

## TECHNICAL REPORT

### Background

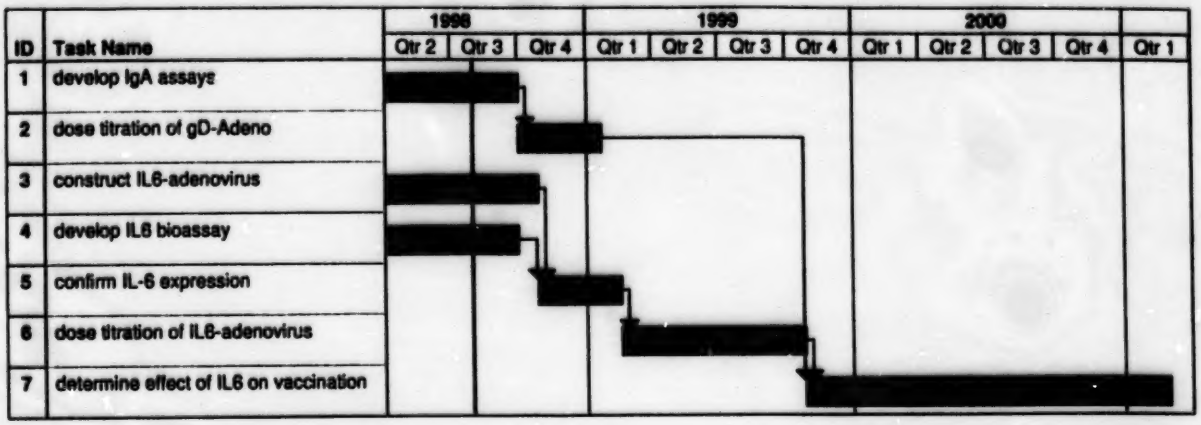
The protection of mucosal surfaces is crucial to the animal's defense against infectious disease. While the skin is relatively impervious to microbial attack, the lining of the gastrointestinal and respiratory tracts are by necessity more permeable, as they must allow for the exchange of gases or the uptake of nutrients. Thus, although the mucous which covers these surfaces provides some physical barrier to infection, these "mucosal" surfaces are much more vulnerable to colonization and invasion by microorganisms. In cattle, common diseases such as shipping fever, scours, enzootic pneumonia and bovine virus diarrhea (BVD) result from viruses or bacteria infecting, or entering the body through, mucosal surfaces. Thus, developing protective immunity at such sites would be very effective in preventing a wide range of infectious diseases. As well as producing more effective immunity, vaccination at mucosal sites avoids injection site lesions, which cost the Canadian beef industry $19 million annually (Van Donkersgoed, 1997, Can Vet J, 38:767).

In practice, however, evoking protective mucosal responses is not so easy. The mucosal immune system, although integrated with the rest of the animal's immune system, has a number of unique features. For instance, one type of antibody, immunoglobulin A (IgA) is much more prevalent in mucosal secretions than in the blood, and has certain features such as resistance to digestive enzymes which make it suitable for that environment. Consequently, mucosal vaccination presents some unique challenges. One important challenge is to develop adjuvants that specifically enhance the mucosal immune response to vaccines. This project will address that challenge, using a strategy which capitalizes on the body's own method of action. The immune system coordinates its response to infection by the timely production of a number of molecules, collectively known as cytokines or interleukins, which transmit signals between the various cells involved in the response. We intend to augment the immune response to a vaccine by administering one of these cytokines, interleukin (IL) 6, with the vaccine. IL6 is an essential factor for the production of IgA, the type of antibody most frequently found in mucosal sites. As well, there is the potential for IL6 to serve as a vaccine adjuvant. Administration of IL6 plus antigen resulted in increased numbers of antibody-containing cells in intestinal loops of rats, and vaccination of mice with the HA gene of influenza and the IL6 gene resulted in enhanced protection from infection.

A second challenge for mucosal vaccines is finding ways to deliver the vaccine to the mucosal site. One option is to use an innocuous bacteria or virus as a vector to carry the gene for the vaccine antigen. Since the live vector can infect the mucosa, the antigen is produced at that site. A second advantage for live vectors is that cytokine genes can also be inserted into the vector and expressed at the same time and place as the antigen. The bovine adenovirus-3 (BAV-3) has been developed as a vaccine vector at VIDO and provided the vehicle for delivery of the IL6 gene that we have isolated, cloned and expressed. We also have produced antibodies to IL6. Thus, we were well positioned to investigate whether IL6 can enhance the response to mucosal vaccines.

**Specific Aims and Milestones:**

1. develop assays to detect gD antibody and gD antibody secreting cells at mucosal surfaces
2. determine the dose of gD-adenovirus which will elicit a mucosal immune response in young lambs
3. construct an adenovirus vector expressing bovine IL6
4. develop a bioassay for bovine IL6
5. confirm the expression of bioactive IL6 from the IL6-adenovirus
6. determine the dose of IL6-adenovirus which will elicit a biologically detectable response without adverse effects
7. assess the effect of IL6 on the antibody response to a vaccine antigen by comparing the level of antibodies to gD produced in lambs which receive gD alone and lambs which receive IL6 and gD




*Modification to Specific Aims:*

Aim 3 (construction of BAV.IL6) extended from Oct 98 to Mar 99
Aim 4 (bioassay) extended from Sep 98 to July 99
Aim 5 (BAV expression of IL6) extended from Feb 99 to July 99
Aim 7 (in vivo trials for adjuvant effect) did not begin until Qtr 1-2000

**Project Activities**

*AIM 1: Develop antibody and ELISPOT assays to detect mucosal antibody (IgA)*

To measure the IgA antibody response, we obtained monoclonal antibody (M67) to ruminant IgA from Dr. K. Nielsen (Agriculture Canada), and purchased rabbit antisera to sheep IgA from Bethyl Labs. Immunoglobulin was purified from both reagents and conjugated to biotin to facilitate the detection of bound antibody with a strepavidin-enzyme conjugate. Different combinations of these reagents were used to detect IgA in sheep nasal secretions and a bovine reference serum (Figure 1.).

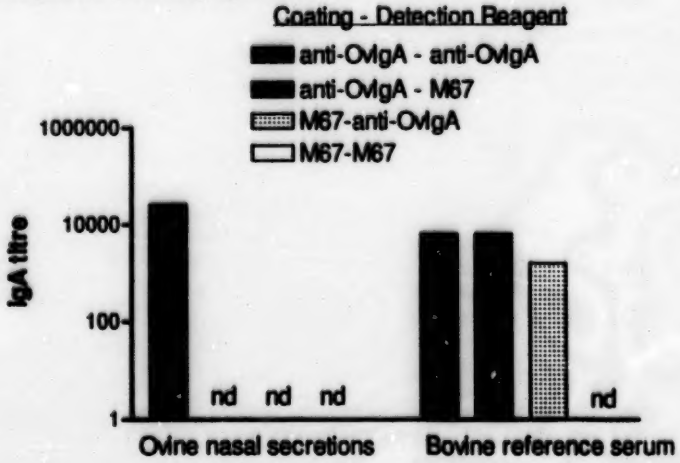



**Figure 1. Detection of Sheep IgA**

*Two reagents reported to be specific for ruminant IgA were used in different combinations as coating and detection antibody, to determine the best combination for a capture ELISA. The reaction with an ovine nasal secretion and a bovine reference serum was expressed as the titre (reciprocal of last dilution to give a color change above background). nd = none detected.*

The M67 monoclonal antibody did not detect sheep IgA. The successful combination of rabbit anti-OvIgA to capture IgA, followed by biotinylated rabbit anti-OvIgA to detect, was used to measure IgA concentration in sheep nasal secretions and colostrum.

*AIM 2: Determine the dose of BAV-3 expressing gD (antigen of bovine herpesvirus-1) that will elicit a mucosal immune response*

An experiment was conducted to determine the dose of BAV3.gD and route of administration-which resulted in a detectable primary mucosal immune response. Sixteen weaned lambs were assigned randomly to 4 groups, and administered adenovirus vector as per the following table.

**Table 1 Dose and route of administration of BAV3.gD**

| | Dose | |
|---|---|---|
| **Route** | $6x10^7$ PFU | $6x\ 10^5$ PFU |
| Subcutaneous | Group SC-Hi | Group SC-Lo |
| Intratracheal | Group IT-Hi | Group IT-Lo |

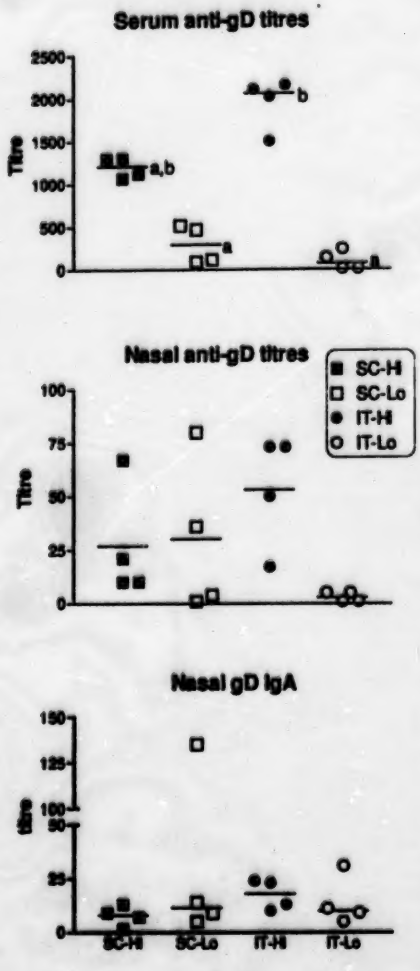



**Figure 2 Antibody responses to bovine adenovirus expressing gD**

*The level of antibody to gD in serum and nasal secretions collected 17 days after immunization with BAV3.gD is shown for each lamb (bar = median). Groups with different letters are significantly different($p<0.05$). Preimmunization titres did not exceed 225 in serum or 4 in nasal secretions. Only one animal made IgA antibodies to gD.*

Serum and nasal secretions were collected at day 0 and day 17 after immunization, and the animals were killed on days 20 & 21 (8 each day) to obtain lung (tracheobronchial) and prescapular lymph nodes, spleen, and jejunal Peyer's patches. Lymphocytes isolated from these tissues were tested for gD-specific lymphoproliferative response (LPR), interferon (IFN)-γ production, and antibody secretion.

*Antibody Responses*

Both route and dose had significant effects on serum anti-gD titres. Antibody levels were elevated in all members of the two high dose groups, with only slight increases apparent in two of the SC-Lo group (Figure 2 ). The antibody level of the IT-Hi group was significantly higher than the two Lo dose groups (Kruskal-Wallis test, $p<0.01$).

In nasal secretions, antibody responses to gD were detected, albeit at low levels, in all members of the two high dose groups (

Figure 2 ). Two of the SC-Lo group also responded. Little of

this nasal antibody response was of the IgA isotype.

*Cell-mediated immunity*

Cell-mediated immunity was assessed by interferon-γ production in response to gD. Interferon production by lung lymph node cells was higher in the IT-Hi group compared to the two SC groups. In all groups, both spleen and jejunal Peyer's patch cells produced interferon, regardless of dose and route.

*Conclusions*

- Immune responses were more consistently demonstrated in the high dose groups; thus, future experiments will use a dose of $10^7$ PFU of BAV3.gD.
- The intratracheal (IT) route was superior to the subcutaneous route in eliciting consistent lung lymph node responses, indicating that this route better induces localized responses in the respiratory tract. The IT route also induced systemic responses such as serum antibody and spleen cell-mediated responses. However, the IT route was not better at inducing nasal antibody, emphasizing the need for adjuvants or other strategies to improve the immune response at mucosal surfaces.

*AIM 3: Construct an adenovirus vector containing the bovine IL6*

Manipulations of the genome of bovine adenovirus-3 were done using the homologous recombination machinery of *E. coli* (Figure 3.). After construction of a full length clone of BAV-3, which is infectious in bovine cells, a transfer plasmid (pBAV.301), in which the E3 region of the BAV genome is deleted, was used to introduce the E3 deletion into the full length clone (again using *E. coli*). Adenovirus can still replicate without the E3 region, and its deletion creates space for the insertion of other genes. Finally, the IL6 gene was cloned into the pBAV.301 plasmid, and the resulting plasmid was used to transfer the IL6 gene into the E3 region of the E3-deleted BAV-3 genomic clone (pFBAV.302). The recombinant BAV-3 genomic clone containing IL6 in the correct orientation in the E3 region was selected by examination of restriction enzyme digestion patterns, and this DNA was transfected into bovine cells. These cells showed evidence of viral infection (cytopathic effect) and produced virus.

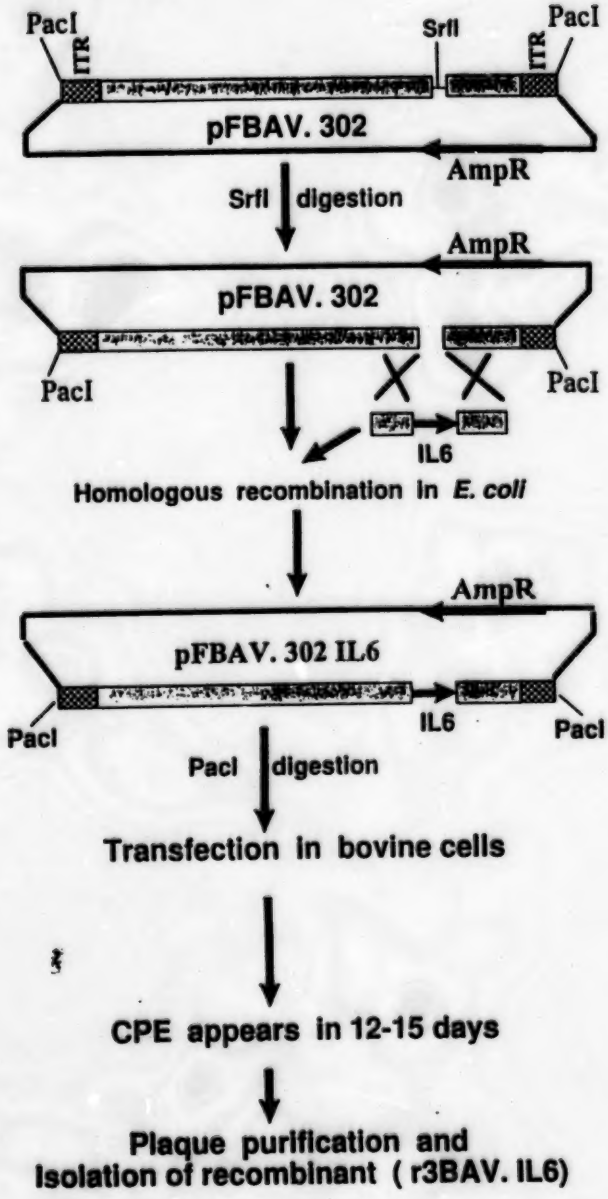



**Figure 3.** *Diagram of construction of bovine adenovirus containing the bovine IL6 gene.*

*A plasmid containing the entire genome of bovine adenovirus-3 with the exception of the E3 region (pFBAV.302) was digested with Srf1 enzyme to open the site where the E3 region has been deleted. The bovine IL6 gene in the transfer plasmid (pBAV.301) was inserted into this linearized plasmid using the homologous recombination machinery of E.coli. The PacI enzyme was used to cut the adenovirus genome from the plasmid and this DNA was transfected into bovine cells. Since this DNA codes for all the required information, new adenoviruses were produced in these cells. By isolating cells infected by a single virus (plaque purification) a homogenous stock of virus can be produced for characterization and confirmation that the IL6 gene remains inserted and intact.*

The first recombinant virus produced unfortunately had a mutation at the end of the IL-6 gene, which resulted in some additional amino acids being added to the molecule (BAV3.IL6x). A second viral construct with the correct gene sequence was produced (BAV3.IL6cs), but the IL6 protein was not secreted out of the infected cell, as detected by immunoprecipitation. A third construct was produced which expresses IL6 and the gD protein of BHV-1 (BAV3.tgDciteIL6)

*AIM 4: Develop a bioassay to confirm the expression of IL6*

Three potential assays were evaluated:

*Hybridoma (7TD1) proliferation.*

This cell line is responsive to picogram amounts of murine and human IL6. While some investigators have used this assay to measure IL6 activity from ruminant sources, others have reported that the cell line is not responsive to ovine IL6 (Ebrahimi et al. (1995) Cytokine, 7:232). Supernatants from cells transfected with the bovine IL6 gene, and shown to contain IL6 protein, did not stimulate proliferation of 7TD1 cells, indicating that this cell line was not responsive to ruminant IL-6.

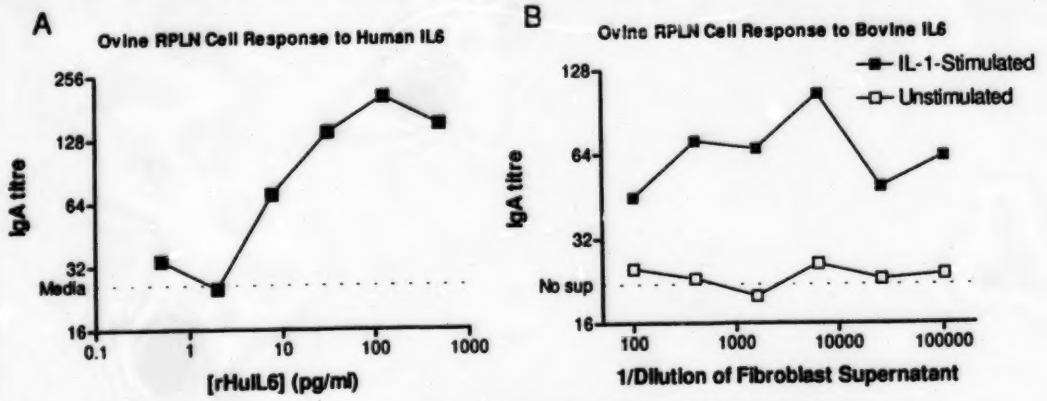
*Induction of alkaline phosphatase on bovine endothelial cells*

Using reports that IL6 induces differentiation of endothelial cells, we developed an assay which measures alkaline phosphatase production by cultured bovine pulmonary endothelial cells in response to IL6. Supernatants from cells transfected with the bovine IL6 gene, discussed above, were incubated with endothelial cells for 5 days. Alkaline phosphatase production, as assessed by a change in color of a phosphatase sensitive substrate, was induced by recombinant human IL6 and the IL6-transfected cell supernatant, but not the null-transfected cell supernatant. Alkaline phosphatase also was induced when endothelial cells were exposed to culture supernatants of fibroblasts treated with recombinant bovine IL-1 (reported to induce IL6 production by fibroblasts). However, the effect of the fibroblast supernatants could not be completely abrogated by the addition of anti-IL6. Thus, other mediators produced in cell culture can also influence alkaline phosphatase levels on endothelial cells, and this assay was not suitable for testing supernatants from BAV vectors.

*In vitro antibody production.*

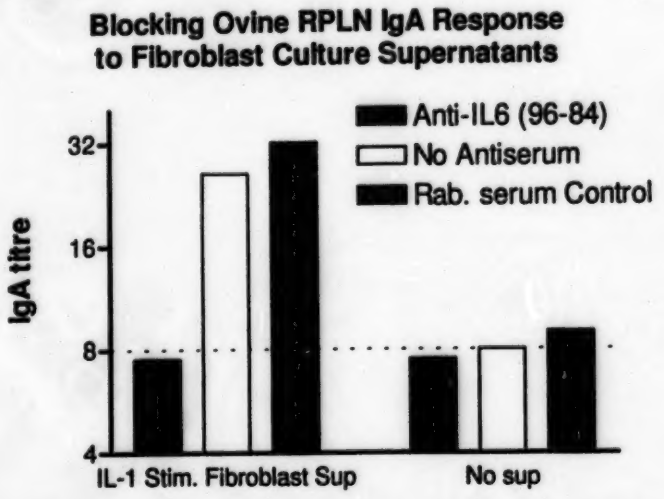
In the assay described by Ebrahimi *et al.*, ovine IL6 increased the amount of antibody produced by peripheral blood mononuclear cells (PBMC) stimulated with pokeweed mitogen. As part of other studies at VIDO, we found that retropharyngeal lymph nodes (RPLN) of sheep contain a high frequency of B cells which produce IgA. These cells responded to rHuIL6 (Figure 4A) with increased production of IgA. Culture supernatants from bovine fibroblasts stimulated with rBoIL1, to induce production of IL6, also caused the RPLN to produce increased amounts of IgA (Figure 4B). The causative component of these supernatants was confirmed to be bovine IL6 as the response could be completely blocked by the addition of anti-IL6 (Figure 5).

**Figure 4 Ovine Retropharyngeal Lymph Node Cell Response to IL6**




*Ovine retropharyngeal lymph node cells were incubated for 6 days in the presence of recombinant human IL6 or supernatants from bovine fibroblasts stimulated with rBoIL1. The amount of IgA produced by the cells was determined by ELISA and the level of production by unstimulated cells is shown by the dotted line.*

**Figure 5 Anti-IL6 Blocks IgA Inducing Effect of Bovine Fibroblast Culture Supernatants**



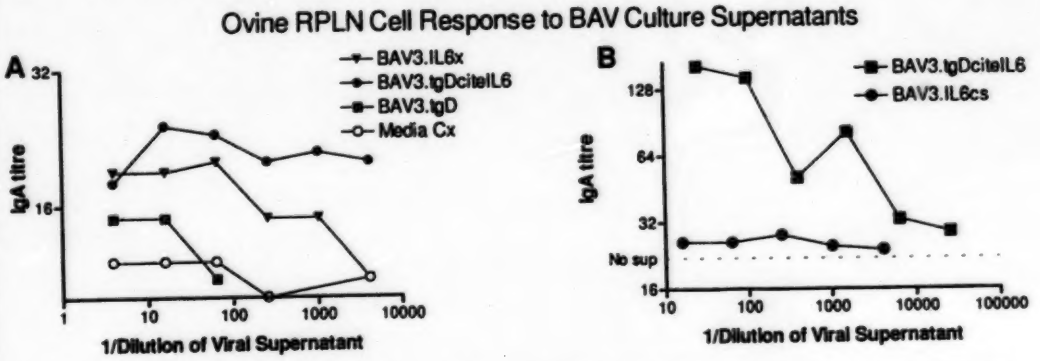

*Ovine retropharyngeal lymph node cells were cultured for 6 days in the presence of supernatants from bovine fibroblasts stimulated with rBoIL-1. Rabbit anti-IL6 (1/100 final concentration) or an equivalent amount of normal rabbit serum was added to some cultures. The amount of IgA produced by the RPLN cells was determined by ELISA. The dotted line represents the amount of IgA produced by the RPLN cells without any additions to the culture media.*

*AIM 5 Confirm the expression of bioactive IL6 from the IL6-adenovirus*

To confirm whether BAV3 vectors which contained the IL6 gene produced bioactive IL6, supernatants from infected cells were added to cultures of ovine RPLN cells, and the level of IgA production was determined (Figure 6). BAV3.tgDciteIL6-infected cells produced the highest levels of bioactive IL6. The IL6 protein produced by BAV3.IL6x, although differing slightly in structure from natural IL6, was still bioactive. The level of IL6 activity from BAV3.IL6cs was quite low, confirming immunoprecipitation results that little protein is released from infected cells into

the culture media. The effect of supernatants from BAV3.tgDciteIL6 were blocked by the addition of anti-IL6.

**Figure 6 Production of Bioactive IL6 by BAV3 Vectors**




*Ovine retropharyngeal lymph node cells were cultured in the presence of supernatants of MDBK cells infected with different bovine adenovirus constructs expressing the bovine IL6 gene in two separate experiments. The production of IgA by RPLN was quantitated by ELISA.*

*AIM 6 Determine the dose of IL6-adenovirus which will elicit a biologically detectable response without adverse effects*

Two trials were conducted to address this aim. Lambs were infected intratracheally, and in one trial also intranasally, with one of 3 vectors containing IL6: BAV3.IL6x, BAV3.IL6cs and BAV3.tgDciteIL6 . Control lambs received an equivalent amount of BAV3.tgD. The vectors and amounts administered are described in Table 1. We attempted to increase the amount of virus administered in the second trial, but the BAV3.tgDciteIL6 propagates poorly and we did not increase the amount of that virus administered.

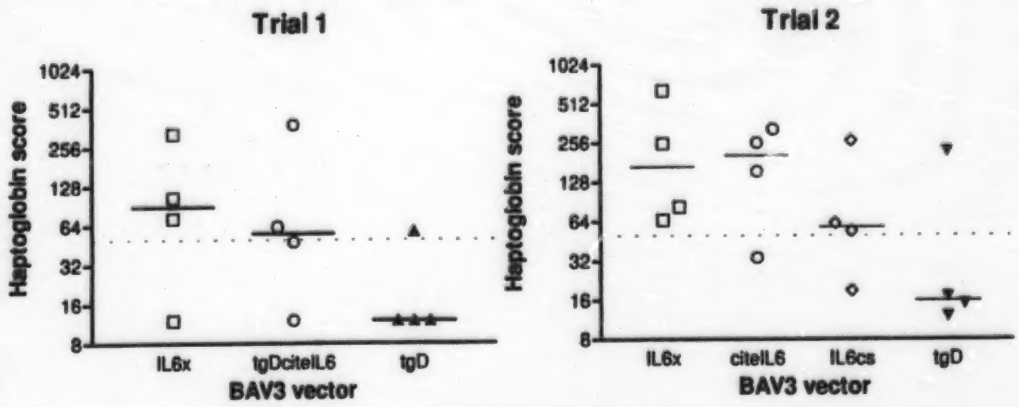
**Table 2 Bovine adenovirus vectors used to deliver IL6 in vivo**

| | Trial 1 - BAV/lamb | Trial 2 - BAV/lamb | | |
|---|---|---|---|---|
| | | Intratracheal | Intranasal | Total |
| BAV3tgD | $3.90 \times 10^8$ | $2.10 \times 10^9$ | $4.15 \times 10^8$ | $2.52 \times 10^9$ |
| BAV3tgDcite IL6 | $1.45 \times 10^8$ | $2.41 \times 10^7$ | $3.90 \times 10^6$ | $2.80 \times 10^7$ |
| BAV3IL6x | $1.12 \times 10^8$ | $2.58 \times 10^9$ | $4.16 \times 10^8$ | $3.00 \times 10^9$ |
| BAV3IL6cs | | $1.50 \times 10^{10}$ | $2.42 \times 10^9$ | $1.74 \times 10^{10}$ |

*Acute phase responses*

Infection with the BAV vectors induced only a very mild fever, with only a tendency for the BAV3.tgDciteIL6-infected lambs to have a slightly higher increase in temperature at 24 to 30 h after infection.

**Figure 7 Induction of the acute phase protein haptoglobin**




*To monitor the acute phase response, serum haptoglobin concentration was determined daily. The sum of daily levels for days 1 to 6 post infection was used as a score of the magnitude of the acute phase response. A score of 50 was used as a threshold to indicate a biologically significant response.*

In the first trial, haptoglobin was elevated in 3 of 4 lambs in both BAV3.IL6x and BAV3.tgDciteIL6 groups and only 1 lamb in the BAV3.tgD group (Fig 7). In the second trial, more lambs had detectable levels of haptoglobin, but using a cumulative haptoglobin score of 50 as indicating an acute phase response, all 4 animals in the BAV3.IL6x group, and 3 of 4 animals in the BAV3.IL6cs and BAV3.tgDciteIL6 groups had an acute phase response, while only 1 animal in the control group did.

*Altered lymphocyte trafficking*

For Trial 1, no differences between groups were observed in total WBC or differential leukocyte counts. Histopathology revealed noticeable lymphocyte hyperplasia in the lungs, but scoring of lymphocyte infiltration, done by Dorothy Middleton (Veterinary Pathology, WCVM), indicated no significant differences between groups. Thus, the changes seen may have been due to the response to adenovirus or were preexisting changes.

In both trials, the percentage of lymphocytes in bronchoalveolar lavages (BAL) ranged from 5% to 23%, but all groups had a similar mean percentage.

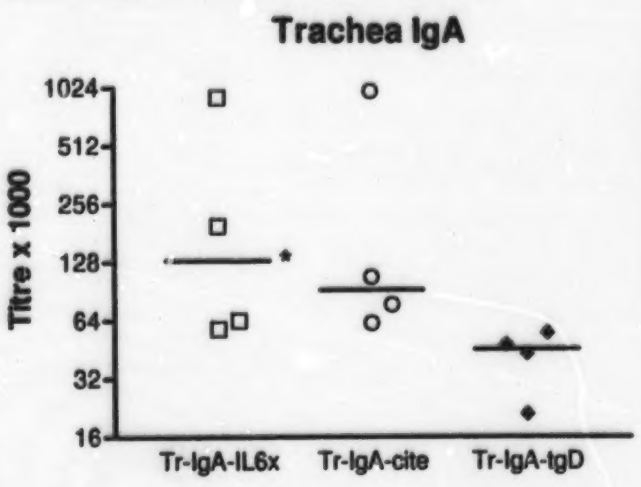
*Enhanced Ig (total and IgA) production*

There were no differences in the frequency of antibody secreting cells in mediastinal lymph nodes, or the level of antibody production *in vitro* by isolated lymph node cells.

In Trial 1, the IL6 groups tended to have higher concentrations of antibody in bronchoalveolar lavage (BAL) fluid and tracheal mucous (Fig 8), with tracheal IgA being significantly higher ($p<0.02$). Serum and nasal antibody levels did not show any clear differences between groups.

In Trial 2, there were no apparent differences in IgA levels in the lung fluid.

**Figure 8 IgA Levels In Tracheal Mucous**




*4 lambs were infected with each BAV3 vector for 7 days. Mucous was collected from the trachea and the IgA content of the fluid was determined by ELISA. The level of IgA for each lamb is shown as a point and the median level for the group is shown by the bar.*

Lambs had some level of preexisting antibody to BAV3 at the time of challenge, and this increased approximately 20 fold by day 7, indicating that there was active immunity to BAV3 present in the lambs. There were no difference between groups in this response.

In summary, we expected IL6 expression to result in an acute phase response, increased lymphocyte infiltration into the lung and possibly increased levels of antibody or antibody secreting cells. Lambs administered BAV3.IL6x or BAV3.tgDciteIL6 tended to have indications of increased temperature and acute phase protein production. In one trial, they also had higher levels of IgA in tracheal and lung fluid. However, this trend was not seen with the frequency of antibody secreting cells in the draining lymph node, nor was there an effect on the level of antibody in nasal secretions. There also was no indication of lymphocyte infiltration into the lungs, as had been seen with rodents administered IL6-expressing adenovirus. Thus, although the two IL6 containing BAV do produce bioactive IL6, the level of expression, or the replication of the vector is low so that induced responses are not dramatic.

*AIM 7 Assess the effect of IL6 on the antibody response to a vaccine*

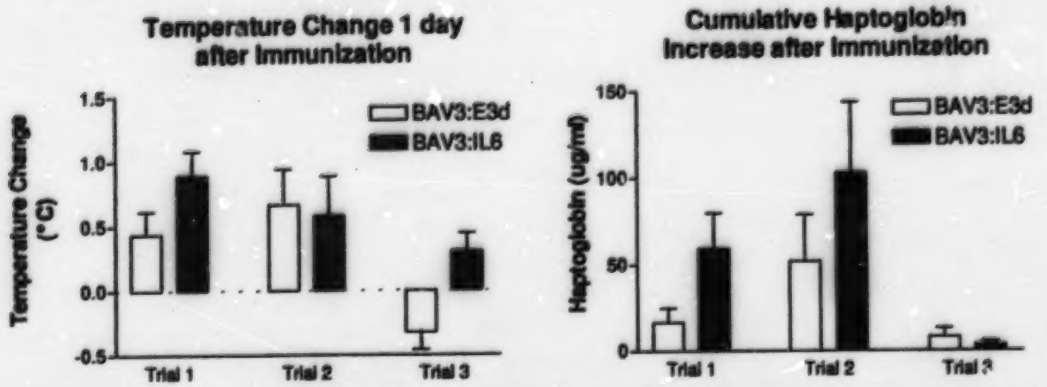
To evaluate the effect of IL6 as a mucosal vaccine adjuvant, 2 groups of lambs were administered BAV3 expressing the gD protein from bovine herpesvirus-1 (the antigen). One group also received BAV3 expressing IL6 (BAV3.IL6x). The immune response to gD in this group was compared to the control group which received an equivalent amount of BAV3.E3d (with the E3 region deleted and no foreign gene inserted). Since the number of animals required to assess this objective could not all be done in a single experiment, three replicate trials were completed. In each trial, 2 groups of 8 lambs (about 10-12 weeks old) were infected intratracheally and intranasally with BAV3 vectors.

**Table 3 Experimental treatments to assess IL6 effect on vaccine responses**

| Group | Adjuvant | Antigen |
|---|---|---|
| Vector Control | BAV3.E3d ($2x\ 10^9$) | BAV3.gD ($10^7$) |
| IL6 | BAV3.IL6x ($2x\ 10^9$) | BAV3.gD ($10^7$) |

Acute phase responses were monitored to determine if the IL6 expressed by the BAV vector stimulated systemic responses. A transient increase in body temperature was observed in most lambs one day after immunization. In two of three trials, this increase was higher in the IL6-treated group, but overall there was not a significant effect of IL6 on temperature. In addition to temperature, the serum concentration of the acute phase protein, haptoglobin was monitored. A mild increase in haptoglobin occurred following immunization. Again, in two of three trials, this increase was higher in the IL6-treated group but overall there was not a significant effect of IL6 on haptoglobin response.

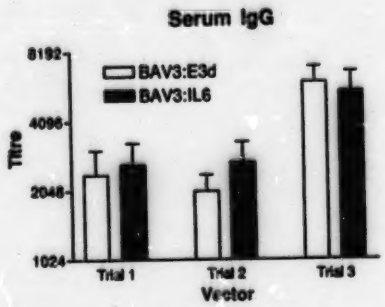
**Figure 9 Acute phase responses following immunization**



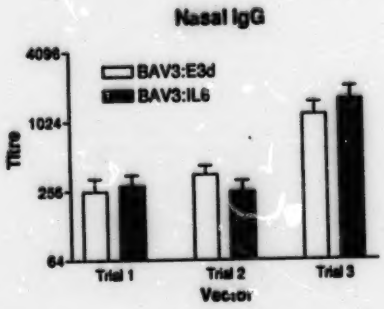

Lambs (n=8/group/trial) were immunized with BAV3 expressing tgD plus either BAV3 expressing IL6 (BAV3:IL6) or the null vector (BAV3:E3d) in three separate trials. A transient increase in body temperature was observed in most lambs one day after immunization. The increase in temperature over the pre-immunization baseline for each individual was calculated and the mean (+/- SEM) for the group is shown. As well, the mean cumulative increase in haptoglobin concentration over the first four days post immunization is shown.

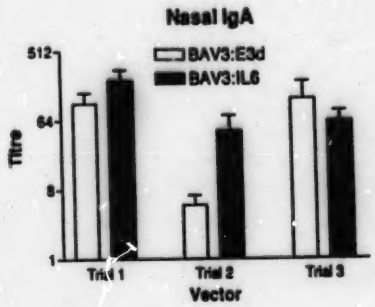
Primary antibody responses to tgD were measured in serum, nasal secretions, tracheal mucous and lung wash fluid following immunization with BAV3 expressing tgD plus either BAV3 expressing IL6 (BAV3:IL6) or the null vector (BAV3:E3d). In all cases, in two of three trials the group coimmunized with BAV3:IL6 had a higher antibody response. However, the effect of IL6 never reached statistical significance, even in the case of tracheal IgG in which BAV3:IL6 immunized groups had a higher response in all trials.

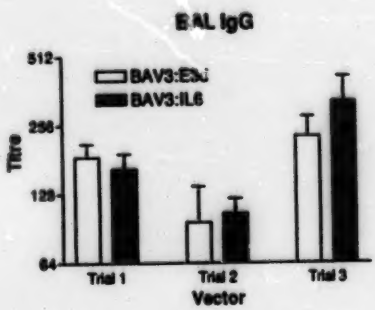
**Figure 10 Antibody responses to gD**

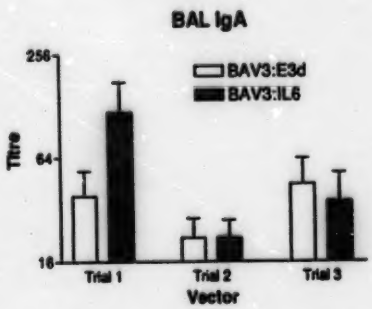


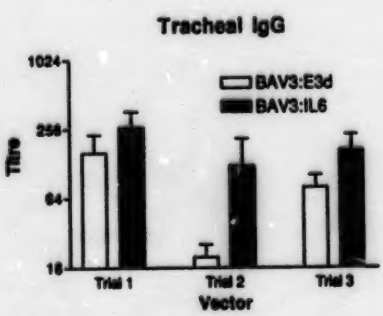

Lambs (n=8/group/trial) were immunized with BAV3 expressing tgD plus either BAV3 expressing IL6 (BAV3:IL6) or the null vector (BAV3:E3d) in three separate trials. The amount of antibody specific for gD was determined in serum, nasal secretions, tracheal mucoud, and lung wash fluid. For the samples from the respiratory tract, the amount of IgA specific for gD was determined as well.

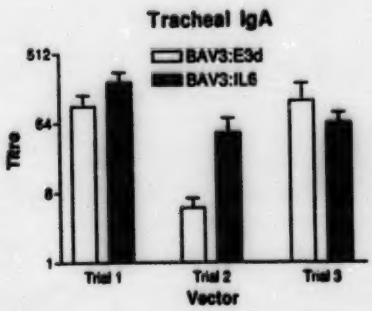


Thus, these trials did not establish the hypothesis that coadministration of IL6 with an antigen would augment the immune response to that antigen. In all measurements, the IL6-treated group had better responses in at least two of three trials. However, this slight IL6 effect was too small to become significant given the animal-to-animal and trial-to-trial variation. In addition, that effect is too small to be of practical and commercial significance.

**Conclusions**

Mucosal vaccines remain attractive for reasons of effectiveness in preventing infection and non-invasive (no tissue damage) administration. However, challenges still exist in finding good adjuvants and appropriate methods of delivery for mucosal vaccines.

In this project, we:

- constructed an adenovirus vector containing the bovine IL6 gene
- developed a bioassay to confirm the expression of IL6
- determined the dose of IL6-adenovirus which elicited a biologically detectable response, without adverse effects, in young lambs
- assessed the effect of IL6 on the antibody response to a vaccine antigen.

The work was conducted as per the research plan, with only minor modifications in time lines. We constructed a BAV3 vector which produced bioactive IL6 in vitro and could elicit IL6 dependent responses in vivo, albeit at a low level. However, these trials did not establish the hypothesis that coadministration of IL6 with an antigen would augment the immune response to that antigen. Over the course of this project, we found that the BAV3 vector replicates less well in sheep than cattle. Thus, the amount and duration of IL6 expression was less than projected. Thus, one question arising from these studies is whether IL6 is present in sufficient amounts or for a sufficient period to induce heightened immune responses. Whether enhancing the level or duration of IL6 presence could increase the adjuvant effect is a question for the future, but delivery of the appropriate amount of cytokine at the appropriate time remains a key issue to exploiting cytokines for immunostimulatory purposes.

**Information of benefit to producers**

Vaccines that are delivered to the mucosal sites, such as the respiratory or gastrointestinal tracts, may be more effective in preventing disease since they stimulate immunity at the surfaces where many viruses and bacteria start their infection. As well, intranasal or oral vaccines have no injection site lesions, improving carcass quality.

In this project, we proposed to improve the immune response to mucosal vaccines by administering a cytokine, or natural regulator of the immune system, interleukin (IL6), with the vaccine. We confirmed that mucosal vaccines could be effective in stimulating immune responses. However, our results also highlighted the need for further research to define the conditions required to harness the immunostimulatory potential of cytokines.

**Achievements, Papers, and Presentations**

A poster presentation was submitted to the Canadian Society for Immunology Spring Meeting, Mar 5-8, 1999, at Lake Louise, AB, entitled,

"DETECTION AND MEASUREMENT OF BOVINE INTERLEUKIN-6"

D.L. Godson[1], Y.I. Popowych[1], S.K. Tikoo[1], M.A. Morsey[2], & P.J. Griebel[1]

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The support of the Sask ADF was acknowledged in the abstract of the presentation.

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